

# **INTERNSHIP REPORT**

Submitted to

**SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY**

In partial Fulfilment for the Award of The Degree Of

**BACHELOR OF SCIENCE**

In

**MEDICAL LABORATORY TECHNOLOGY**

By

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**DEPARTMENT OF MEDICAL LABORATORY TECHNOLOGY**

**SCHOOL OF ALLIED HEALTH SCIENCES**

# **SATHYABAMA**

**INSTITUTE OF SCIENCE AND TECHNOLOGY**

**(DEEMED TO BE UNIVERSITY)**

Accredited with Grade "A++" by NAAC

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**APRIL 2024**



**SATHYABAMA**

INSTITUTE OF SCIENCE AND TECHNOLOGY  
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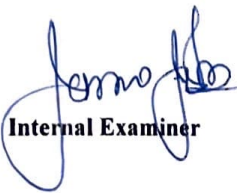
This is to certify that this internship report is the bonafide work of **MOHD TAHIR A** who have done the project work as a team and carried out the training program at **SATHYABAMA GENRAL HOSPITAL** and **LIONS BLOOD BANK AND RESEARCH FOUNDATION EGMORE** under our supervision from August 2023 to April 2024.

  
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## DECLARATION

I, **A. Mohd Tahir (41737009)**, hereby declare that the internship trainings done by me at **LIONS BLOOD BANK AND RESEARCH FOUNDATION at EGMORE** under the guidance of **Ms. D. JASMINE PRIYA** and "**SATHYABAMA GENRAL HOSPITAL at Sathyabama**" under the guidance of **Dr. JAMES JOHN** at Sathyabama Institute of Science and Technology is submitted in partial fulfilment of the requirements for the award of Bachelor of science degree in **MEDICAL LABORATORY TECHNOLOGY**.

DATE: 12/4/24

PLACE: CHENNAI

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I am worked in the CHENGALPATTU MEDICAL COLLEGE – CHENGALPATTU DISTRICT, Histopathology laboratory during my internship training period on 01<sup>st</sup> November 2023 to 15<sup>th</sup> November 2023 from 10am to 4pm.

## **HISTOPATHOLOGY**

### **SAMPLE RECEIVING AREA**

- Tissue specimen received in the laboratory have a request form that lists the patient information, history & description of the site of origin.
- The specimen is accessioned by giving them a number that will identify each specimen for each patient

### **SAMPLE RECEIVING CRITERIA**

- Specimens only requiring transfer from container to tissue cassette.
- Specimens requiring transfer but with standard sampling, counting, weighing or slicing.
- Simple dissection required with sampling needing a low level of diagnostic assessment and/or preparation.
- Dissection and sampling required needing a moderate level of assessment.
- Specimens requiring complex dissection and sampling methods
- Brain: Submit either without sectioning or with coronal (divides into front and back) sections at 1–2-inch intervals.
- Spinal cord: Submit either intact (with the dura mater opened) or in segments at least one inch in length.
- Intestines: Submit in segments 1-2 inches in length.
- Lymph nodes: Section once in sagittal plane (divides into left and right halves).
- Large masses: Incise to allow penetration by formalin.
- The ratio of formalin to tissue should be at least 20 parts formalin to 1 part tissue. Multiple tissue types from the same animal can be submitted in the same container as long as this ratio is maintained.



Figure- 1

## GROSSING

Grossing of specimen is important stage in surgical pathology.

Gross description provides information that aides the pathologist in making a diagnosis during microscopic evaluation of the tissue.

It includes cutting smaller pieces of tissue from the entire gross specimen submitted to the laboratory.

It involves:

- Accurate naked eye description of intact specimen
- method of sectioning
- Correct Gross examination of cut surface
- Selection of proper tissue blocks for microscopy
- Instructions for embedding & block making.

Histopathology specimens are a vital in-patient care.

They not only establish tissue diagnosis, but are crucial in clinical management

## GENERAL PRINCIPLES OF GROSSING

- ✓ Specimen identification
- ✓ Identify all the anatomical structures present
- ✓ Orientation markers should be identified
- ✓ Measurements: Length, breadth, height
- ✓ Weight-especially parenchymatous organ
- ✓ Examine the external surface.
- ✓ Cut all the organs at intervals of 1cm thickness
- ✓ Describe cut surface, identify pathologic process
- ✓ If suspected lesion is present, measure, describe with color & consistency.
- ✓ Surgical margins
- ✓ Histological sections



Figure-2



Figure-3

# TISSUE PROCESSING

## INTRODUCTION:

Tissue processing: The aim of tissue processing is to embed the tissue in a solid medium firm enough to support the tissue and give it sufficient rigidity to enable thin sections to be cut, and yet soft enough not to damage the knife or tissue

## STEPS OF TISSUE PROCESSING

### 1.FIXATION:

- Fixation is the foundation for the subsequent stages in the preparation of tissue sections, up to the making of diagnosis.
- The process of autolysis and bacterial attack should be prevented. Tissues should not change shape or volume and they should be left in a condition which subsequently allows clear staining of sections.
- At the same time, tissues should be as close to their living state as possible without loss or rearrangement. The choice of fixative for routine histology varies/differs for the different groups of chemical substances found in tissues.
- The most commonly used fixative in histopathology is 10% neutral buffered formalin.
- The time required for correct, adequate and complete fixation varies from 6-48 hours depending on the size, type or nature of the tissue.

### DEHYDRATION:

To remove fixative and water from the tissues and replacing them with dehydrating fluid

### CLEARING:

Replacing the dehydrating fluid with a fluid that is totally miscible with both the dehydrating fluid and the embedding medium

### IMPREGNATION:

Replacing the clearing agent with the embedding medium.



Figure-4



Figure-5

## EMBEDDING

### INTRODUCTION:

Embedding is the process in which the tissues or the specimens are enclosed in a mass of the embedding medium using a mold. Since the tissue blocks are very thin in thickness, they need a supporting medium in which the tissue blocks are embedded. This supporting medium is called embedding medium. Various embedding substances are paraffin wax, celloidin, synthetic resins, gelatin, etc.

The choice of embedding media depends upon

- Type of microscope
- Type of microtome
- Type of tissue eg. hard tissue like bone or soft tissue like liver biopsy

**Paraffin wax** with a higher melting point (56 to 62°C) is used for embedding. The molten wax is filtered inside the oven through a coarse filter paper into another container. This will protect the knife edge

### OTHER TYPES OF EMBEDDING MEDIA:

- **Celloidin media:** Celloidin is a purified form of nitrocellulose. It is used for cutting hard tissues.
- **Gelatin:** Its melting point is less than the melting point of agar. Gelatin may be used when frozen sections are required on friable and necrotic tissues.
- **Carbowax:** It is a water-soluble wax. Therefore, tissues are directly transferred to water-soluble wax after fixation and washing.
- **Methacrylate:** It is easily miscible with alcohol and gives a clear and hard block when polymerized. Polymerization takes place in the presence of a catalyst. Any trace of water causes uneven polymerization and formation of bubbles in the block around the tissue.
- **Agar embedding:** It is mainly used in double embedding. Multiple fragments and friable tissue may be impregnated in one block when sectioning on the cryostat. Another use of agar embedding is for FNAC specimens.
- **Paraffin Embedding:** Paraffin, also known as paraffin wax, is a traditional and widely employed embedding agent in histology laboratories. Its main components are long-chain hydrocarbons derived from petroleum.



Figure-6



Figure-7

## SECTION CUTTING

### INTRODUCTION

In histopathology, section cutting refers to the process of slicing tissue samples into thin sections for microscopic examination. This is a crucial step in studying tissue morphology and identifying any abnormalities or diseases.

### MICROTOMES

Mechanical device designed for accurate cutting of thin, uniform tissue sections for microscopic examination. An advancing mechanism moves the block towards the cutting tool for a predetermined distance

- The tissue moves vertically towards the cutting tool
- Thickness is graduated in microns
- A ribbon of sections is produced
- Ideal thickness: 4-6 microns

### GOOD SECTIONING CRITERIA

- 1- Appropriate microtome and appropriate knife
- 2- Well prepared knife
- 3- Perfect setting includes:
  - ✓ Relation of the knife to the object
  - ✓ Tilt of inclination of the knife
  - ✓ Angle clearance which depends on the profile of the knife, it governs the angle of slant.
- 4- Proper processed tissue
- 5- The skill of the technologist
- 6- The rate of cutting
- 7- The temperature of the atmosphere and the moisture
- 8- The orientation of the tissue block.



Figure-8

## STAINING

### INTRODUCTION

Staining is the process of applying dyes on the sections to see and study the architectural pattern of the tissue and physical characteristics of the cell.

Hematoxylin and Eosin (H&E) staining is used routinely in histopathology laboratories as it provides the pathologist/researcher a very detailed view of the tissue

### H & E STAINING

1. Purpose: To Stain tissue Sections & Histopathology
2. Primary Sample
  - Type --All Sections Cast by Histopathology.
  - Container-- Glass/pvc, which can hold 100ml
  - Quantity—Maximum 100 ml \minimum 75 ml
  - Stability: Prepared at least 3 months earlier
  - Rejection Criteria --Single Section / Partial Section/ floated section
3. Safety precautions: Use Gloves, Apron & Goggles
4. Methodology
  - ✓ Keep Sections/Slides in incubator 50-55°C to 15minutes
  - ✓ Take the Stile to Xylene, Immerse it xylene for 1 hour
  - ✓ Check if war got removed, by naked eye
  - ✓ Immerse slide in graded alcohol-100%, 90%, 80%,70% for 1minutes each
  - ✓ Take to water & Wash – 1 minute
  - ✓ Immerse in Container having Hematoxylin for 25 minutes.
  - ✓ wash in Tap water.
  - ✓ Decolorize in 1% acid alcohol-just 1 dip
  - ✓ Blueing by running tap water-. 10minutes
  - ✓ Dip in Eosin 1%--3 dips
  - ✓ Wash in Tap Water.
  - ✓ Air Dry

- ✓ Mount the Section using DPX mount

## PAP STAINING

It is a histological and cytopathological staining technique used to differentiate cells in a smear preparation. Several specimens can be used to prepare the pap smear depending on the screening infection, including sputum, urine, cerebrospinal fluid, abdominal fluid, tumor biopsies, synovial fluid, fine needle aspirates, pleural fluids

## PROCEDURE

- Fix the smear with 95% Ethanol 15 minutes
- Rinse in tap water
- Add the Harris Hematoxylin dye for 1-3 minutes
- Rinse in tap water or Scott's tap water
- Dip the preparation in 95% Ethanol 10 dips
- Add orange G-6 stain for 1.5 minutes.
- Dip in 95% Ethanol 10 dips
- Add Eosin dye; EA-50, or Modified EA-50, or EA-65 stain for 2.5 minutes.
- Dip in 95% ethanol 10 dips, 2 changes
- Add 100% Ethanol for 1 minute
- Clear in 2 changes of xylene, 2 minutes each
- Mount with permanent mounting medium



Figure- 9

## HAEMATOLOGY

### COMPLETE BLOOD COUNT (CBC)

A complete blood count (CBC) is an important and readily available investigation that focuses on Red Blood Cells, White Blood Cells and Platelets, and their various parameters. It can help to serve as a screening test for many disorders and as a prognostic or follow up tool. It is a Panel of tests that examine different components of the blood.

CBC values

- ✓ RBC count
- ✓ Hemoglobin
- ✓ Hematocrit
- ✓ RBC indices
- ✓ WBC count and differential
- ✓ Platelet count

### THE SIGNIFICANCE OF CBC

- ❖ Find the cause of symptoms such as fatigue, weakness, fever, bruising, or weight loss
- ❖ Diagnosis of anemia
- ❖ Estimation of blood loss
- ❖ Diagnosis of polycythemia
- ❖ Find an infection
- ❖ Diagnosis of blood diseases as leukemia
- ❖ Response to drug or radiation treatment
- ❖ Screening before surgery
- ❖ Abnormal count of certain types of cells



Figure- 10

## ERYTHROCYTE SEDIMENTATION RATE (ESR)

- The rate at which erythrocytes settle down at the bottom of the tube at 1st one hour is regarded as erythrocyte sedimentation rate.
- ESR is a commonly used nonspecific test in routine clinical practice
- Easy to perform, widely available and inexpensive
- Widely used as screening test
- Also used as a monitoring tool for response to treatment in conditions in which it is raised (tuberculosis, autoimmune diseases...etc.)
- ESR is not diagnostic of any particular disease, but rather is an indication that a disease process is ongoing and must be investigated

## FACTORS INCREASING ESR

- ✓ ↓Htc, blood viscosity↑
- ✓ ↑ concentration of fibrinogen (i.e., pregnancy, vascular diseases, heart diseases), haptoglobin, lipoproteins, immunoglobulins
- ✓ Macrocytic RBC & Extreme elevation of WBC count (leukemia)

## FACTORS DECREASING ESR

- ✓ ↑ Htc, ↑ albumin concentration
- ✓ Change in the RBC shape (i.e., sickle-cell anemia, poikilocytosis - nonuniformity of shape)



Figure- 11



Figure-12

## BLOOD SMEAR MAKING

### PROCEDURE (THIN BLOOD FILM)

- Place a drop of blood in the center line of slide 1 cm from one end.
- Place the spreader in front of glass slide at an angle of 30°.

- Move the spreader back, so that it touches the blood drop. Blood will spread along the edge of spreader by Capillary action.
- Push the spreader forward along the length of slide by rapid but straight movement.
- Then allow the smear to dry in the air before staining.
- When dry, patient's name, number and other identifications may be written on the slide with a diamond marker or pencil.

#### PROCEDURE (THICK BLOOD FILM)

These are widely used in the diagnosis of Hemiparasites, particularly malarial parasites. Generally, the blood films should be made about 10 times thickness of normal smears.

- ❖ Place a drop of blood on a clean slide and spread it with the corner of another slide until the printed matter is just visible through the smear.
- ❖ Allow to air-dry and label the slide properly.

#### CRITERIA FOR GOOD BLOOD SMEAR-

- ❖ It should cover entire width and 2-3 part of slide.
- ❖ Tongue shaped (no tail)
- ❖ Uniform thickness
- ❖ No cross striations or air gaps

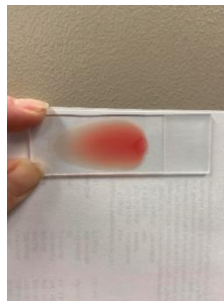


Figure-13

#### RETICULOCYTE SMEAR

- ✓ Put 2 drops of new methylene blue in a test tube
- ✓ Add 2 drops of well mixed EDTA blood to the tube.
- ✓ Mix well.
- ✓ Incubate the mixture for 20 minutes at the temperature of 37 degree in the incubator.
- ✓ Mix the solution again.
- ✓ Prepare 2-4 good thin smears.
- ✓ Label and let dry.

Formula:

$$\text{Reticulocyte count} = \frac{\text{No of reticulocytes counted}}{\text{No of red cells counted}} \times 100$$



Figure-14

## BIOCHEMISTRY

### STOOL EXAMINATION

- ❖ Human feces is called as STOOL.
- ❖ Feces is plural of Latin term faex meaning RESIDUE.
- ❖ It is the waste residue of indigestible materials of an animal's digestive tract expelled through the anus during defecation.
- ❖ Meconium is newborn's first feces.

Requirements:

- ❖ Glass slides
- ❖ Cover slips (22 mm)
- ❖ Normal saline
- ❖ Lugol's iodine solution
- ❖ Saturated saline solution
- ❖ Penicillin bulb

Procedure:

Saline preparation:

- ❖ Place a drop of normal saline on a glass slide
- ❖ Take a little fecal material by using a stick and mix with a drop of normal Saline.
- ❖ Place a cover slip.

Result:

- ❖ Cells: Pus Cells, Epithelial cells, Erythrocytes
- ❖ Parasites
- ❖ Crystals
- ❖ Vegetables matter
- ❖ Undigested ingredients.
- ❖ Other findings (Bacteria and yeast)

## URINE ANALYSIS

Routine Examination of Urine is divided in three parts:

- A. Physical Gross Examination.
- B. Chemical Examination.
- C. Microscopic Examination.

### A. PHYSICAL GROSS EXAMINATION

- Volume of Urine- 50 to 200 ml
- Color of Urine- Pale Yellow, white
- Appearance of Urine-Usually clear
- Reaction- Usually acidic pH 4.88 to 7.5 & more than 7.5 -alkaline urine
- Odor of Urine- Aromatic
- Specific gravity of Urine- Varies from 1.003 to 1.060

### B. CHEMICAL EXAMINATION

- Glucose- NEGATIVE
- Bilirubin- NEGATIVE
- Ketone- NEGATIVE
- Specific Gravity-1.016-1.022
- pH- 5.0-8.0
- Protein - NEGATIVE
- Urobilinogen-0.2-1.0
- Nitrite- NEGATIVE
- Blood- NEGATIVE
- Leukocytes- NEGATIVE

### C. MICROSCOPIC EXAMINATION

In microscopic, I examined the various cells like Pus cells, RBCs, Epithelial cells, Triple phosphate Calcium oxalate, Cholesterol and Uric acid.

## **MICROBIOLOGY**

### **CULTURE**

#### **METHODS OF CULTURE:**

- Streak culture
- Lawn culture
- Stroke culture
- Stab culture
- Pour plate method
- Liquid culture
- Anaerobic culture methods

#### **STREAK CULTURE**

- Used for the isolation of bacteria in pure culture from clinical specimens.
- Platinum wire or Nichrome wire is used.
- One loopful of the specimen is transferred onto the surface of a well dried plate..Spread over a small area at the periphery.
- The inoculum is then distributed thinly over the plate by streaking it with a loop in a series of parallel lines in different segments of the plate.
- On incubation, separated colonies are obtained over the last series of streaks.



Figure-15

#### **LAWN CULTURE**

- Provides a uniform surface growth of the bacterium.
- Uses
  - ✓ For bacteriophage typing.
  - ✓ Antibiotic sensitivity testing.

- ✓ In the preparation of bacterial antigens and vaccines.
- Lawn cultures are prepared by flooding the surface of the plate with a liquid suspension of the bacterium.



Figure-16

### STROKE CULTURE

- Stroke culture is made in tubes containing agar slope / slant.
- Uses
  - Provide a pure growth of bacterium for slide agglutination and other diagnostic tests.



Figure-17

### LIQUID CULTURES

Liquid cultures are inoculated by touching with a charged loop or by adding the inoculum with pipettes or syringes.

- Uses
  - Blood culture
  - Sterility tests
  - Continuous culture methods



Figure-18

## MEDIA

### TYPES OF MEDIA

#### Based on their consistency

1. Solid media - contains 2% agar
  - ✓ Colony morphology, pigmentation, hemolysis can be appreciated.
  - ✓ Eg: Nutrient agar, Blood agar
2. Liquid media - no agar.
  - ✓ For inoculum preparation, Blood culture, continuous culture.
  - ✓ Eg: Nutrient broth
3. Semi solid medium - 0.5% agar.
  - ✓ Eg: Motility medium

#### Based on the constituents/ ingredients

1. Simple media / basal media:
  - ✓ Most common in routine diagnostic laboratories
  - ✓ Eg: Nutrient Broth, Nutrient Agar
  - ✓ NB consists of peptone, meat extract, NaCl, water
2. Complex media
  - ✓ Media other than basal media.
  - ✓ They have added complex ingredients such as yeast extract or casein hydrolysate, which consist of a mixture of many chemical species in unknown proportions
3. Synthetic or defined media
  - ✓ Media prepared from pure chemical substances
  - ✓ exact composition is known
  - ✓ Used for special studies, eg. metabolic requirements
  - ✓ Eg: peptone water- (1% peptone + 0.5% NaCl in water)

#### 4.Special media

- ✓ Enriched media
- ✓ Enrichment media
- ✓ Selective media\
- ✓ Indicator media
- ✓ Differential media
- ✓ Sugar media
- ✓ Transport media

### PREPARATION OF MEDIA

#### Preparation of Media

- ✓ Required amount of chemicals is dissolved in distilled water using Erlenmeyer flasks or rimless clean test tubes.
- ✓ pH is adjusted by adding IN of NaOH or N/10 of HCl
- ✓ 15ml capacity of test tube 5ml of liquid medium is taken
- ✓ 250ml flask
- ✓ 100ml of media
- ✓ These are plugged with non-adsorbent cotton and are covered using brown paper.
- ✓ This is kept for autoclaving-sterilization done at 15lbs/inch<sup>2</sup> at a temperature of 121 degree Celsius for 15min
- ✓ Later cooled down and poured in petri plates or test tubes which solidifies to form a medium
- ✓ Important precautions:
- ✓ Prepare the media from dehydrated products in a damp-free environment.
- ✓ To prevent the risk of inhaling fine particles of culture media powder, wear a dust mask while handling media powder.
- ✓ Wash the hands immediately after preparing media.

#### Properties of Media:

- ✓ Support the growth of the bacteria.
- ✓ Should be nutritive (contains the required amount of nutrients).
- ✓ Suitable pH (neutral to slightly alkaline 7.3-7.4).
- ✓ Suitable temperature
- ✓ suitable atmosphere. (Bacteria grow at 37°C).
- ✓ Sterilized.



Figure-19

## SEROLOGY

### WIDAL TEST (CARD METHOD)

A Widal test meaning is a serology blood test that helps detect typhoid or enteric fever in the body.

To prepare for a Widal test, we would require the following objects:

- Patient's serum
- Pipette (lab tool)
- Serum
- S. Antigen ( O, H, AH, BH)
- Slide
- Mix Stick
- Stopwatch

For this test, you will use a slide with 6 reaction circles, marked as O, H, AH, BH, PC and NC. To begin with,

- Put one drop of the patient's serum in four reaction circles, i.e., O, H, AH, BH.
- Add one drop of positive control in the PC circle and one in the NC circle.
- Next, add one drop of O antigen in the O circle, P antigen in the P circle, AH antigen in the AH circle, and BH antigen in the BH circle, respectively.
- Add any antigen, i.e., O, H, AH, BH in both PC and NC.
- Next, mix the serum and antigen in each circle properly so that the mixture doesn't go out of the circle and touch the slide.
- Also, one mixture should not mix with another, as it can influence the test results.
- Finally, rotate the slide in a slow circular motion to ensure a proper mixture of serum and reagent

Once everything is done, you can see the results. If the test is positive, the test will be similar to the PC (+ve control circle), and if it's negative, it will be similar to NC (-ve control circle). In other words, if there is any agglutination, the test results will be positive.



Figure-20

## C REACTIVE PROTEIN (CRP)

- C-reactive protein (CRP) is a blood test marker for inflammation in the body.
- CRP is produced in the liver and its level is measured by testing the blood.
- CRP is classified as an acute phase reactant, which means that its levels will rise in response to inflammation.

## TEST FOR CRP

### PRINCIPLE

The test is based on the reaction between patient serum containing CRP as the antigen & the corresponding antibody coated to the treated surface of latex particle. The coated particles enhance the detection of an agglutinate reaction when antigen is present in the serum being tested.

### PROCEDURE

#### Qualitative Test:

- Bring reagents and specimens to room temperature before use.
- Place one drop (40 µl) of CRP Positive Control on field #1 of the reaction slide. Place one drop (40 µl) of the CRP Negative Control on field #2.
- Using a serological pipette place (40µl) of undiluted test sample to field #3.
- Gently resuspend the CRP Latex Reagent and add one drop to each test field.
- Mix well with the provided stirring sticks.
- Rotate the slide for 2 minutes and read immediately under an oblique indirect light.

## INTERPRETATION

- A negative reaction is indicated by a uniform milky suspension with no agglutination as observed with the CRP Negative Control.
- A positive reaction is indicated by any observable agglutination in the reaction mixture. The specimen reaction should be compared to the CRP Negative Control



Figure-21

## RA FACTOR TEST

### INTRODUCTION

Rheumatoid factor is an antibody that is measurable in the blood with a routine blood test. Rheumatoid factor is actually an antibody that can bind to other antibodies or normal proteins in our blood that are important parts of our immune system. Rheumatoid factor is an antibody that is not usually present in the normal individual. Because rheumatoid factor antibody binds to normal antibodies, it can be generally referred to as a autoantibody. Health-care professionals use the rheumatoid factor test to assist in the diagnosis of rheumatoid arthritis.

### PRINCIPLE

Detection of Rheumatoid factor by using Latex Agglutination Purpose RF-latex is a slide agglutination test for the qualitative and semiquantitative detection of RF in human serum. Latex particles coated with human gamma globulin are agglutinated when mixed with samples containing

### PROCEDURE

1. The reagents and samples are reached to room temperature. The sensitivity of the test may be reduced at low temperatures.
2. Place 50 µl of the sample and one drop of each Positive and Negative controls into separate circles on the slide test.
3. Swirl the RF-latex reagent gently before using and add one drop (50 µL) next to the sample to be tested.
4. Mix the drops with a stirrer, spreading them over the entire surface of the circle.



Figure-22

## HCV TEST

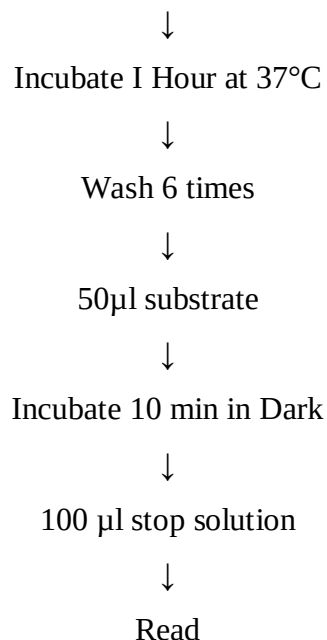
Intended Use Anti-HCV ELISA is an enzyme-linked immunosorbent assay (ELISA) for qualitative detection of antibodies to hepatitis C virus in human serum or plasma. It is intended for screening blood donors and diagnosing patients related to infection with hepatitis C virus.

100 µl sample Diluent  
+  
10µl sample& NC, PC  
↓  
Incubate 45 mins at 37°C  
↓  
Wash 6 times  
↓  
50 µl Assay Buffer  
↓  
50µl conjugate  
↓  
Incubate 30 mins at 37°C  
↓  
Wash 6 times  
↓  
50 µl substrate  
↓  
Incubate 10 mins in Dark  
↓  
100 µl Stop solution  
↓  
Read

## HBsAG TEST

The HBsAg ELISA is an enzyme immunoassay for the qualitative detection of the Hepatitis B surface antigen (HBsAg) in human serum or plasma

25 µl sample Diluent + 75 µl samples NC, PC+ 50µl conjugate in Each well



The presence of HBsAg indicates that the person is infectious, except when it might be transiently positive within 30 days after a dose of hepatitis B vaccine (HepB).

## BLOOD BANK

Blood bank means, a center within an organization grouping and Human donors or an institution for Collection, Cross- matching, Storage, Processing distribution of Whole Human Blood or Blood Products from Selected human the purpose is to meet the challenging requirement of modern-day blood to efficiently Collect blood during emergency.

The main functions of the blood bank are;

- ❖ Recruitment of donors and maintenance of donor's record
- ❖ Collection, preservation; and distribution of blood and blood components
- ❖ Laboratory procedures like, testing for hepatitis B, Hepatitis C, HIV, VDRL for Syphilis and Malaria parasites.
- ❖ Teaching, training and Research
- ❖ Clinical and therapeutic functions like transfusion and day care Service.

## Importance of blood bank;

Blood banking is of particular importance because of its direct tie to helping patients. There are a number of tests that must be performed on blood, and the clinic as a whole would not be able to function without them.

In the laboratory, there are certain tests that must be performed on all donated blood. Blood typing is done to determine the donor's ABO blood group and Rh status; it is then matched to the corresponding patient. Before a patient can receive any donated blood, it is tested to ensure the safety of the blood. Blood is tested for red blood cell antibodies that could cause a reaction in the patient, and units

## Facts about blood banking

- ❖ According to the American Association of Blood Banks as of 2013:
- ❖ About 36,000 units of blood are needed every day.
- ❖ The number of blood units donated is about 13.6 million a year.
- ❖ About 6.8 million volunteers are blood donors each year
- ❖ Each unit of blood is broken down into components, such as red blood cells, plasma, cryoprecipitate AHF, and platelets. One unit of whole blood, once it's separated, may be transfused to several patients, each with different needs.
- ❖ Annually, more than 21 million blood components are transfused

## COMPONENT SEPARATION

### Blood components

- Whole blood
- Packed red cells
- Fresh frozen plasma
- Cryoprecipitate

### Preparation of Red blood cell

Red cells have a higher specific gravity than plasma, thus settle in the lower portion of the bag.

### Procedure

-  Store the bag at 2-6°C till processed

-  Place bag in buckets of refrigerated centrifuge and balance the opposite bags Centrifuge at 5000 x g for 5 minutes at 2-6°C
-  Express approx. 3/4th of the plasma in the satellite bag
-  Keep red cell at 2-6°C and plasma at -30°C

## Preparation of Fresh frozen plasma

Plasma removed from cells within 6-8 hours of collection is rapidly frozen to below

### Procedure

- Collect in triple pack system and store at 4 °C or in air-conditioned room till processed but not for more than 6-8 hours.
- Place bag in buckets of refrigerated centrifuge and balance the opposite bags Centrifuge at 5000g for 5 minutes at 4 °C
- Express approx. (4 / 5th) of the plasma in the satellite bag
- Label the plasma bag and is rapidly frozen within 6-8 hours and the freezing process should not be more than 1 hour at – 70 ° C
- Can be stored at – 30° C or below for 1 year.
- Thaw it in a plasma defroster (Microwave) or place in a plastic over wrap and put it in a 37 °C circulating water bath.

## Preparation of platelets

- Random donor platelet - prepared from 450ml of whole blood
- Single donor platelet - prepared by apheresis

### Procedure

- Collect in triple pack system and store at room temperature at 20-22°C for less than 6 hours.
- Place bag in buckets of refrigerated centrifuge and balance the opposite bags Centrifuge at 2000 × g for 3 minutes at 20-24°C
- Express approx. 4/5th of the platelet rich plasma in the satellite bag
- Centrifuge the bag with PRP and another satellite bag at 20-40°C at 5000 xg for 5 minutes
- Express supernatant platelet poor plasma into another empty bag and leave approx 50 ml of plasma.

## Purpose of Blood Separation

Each blood cell type has its own unique purpose and function. Separating the various blood components allows scientists to analyse specific cell types. Some blood components can be used to detect diseases, while others can provide a more comprehensive understanding of the human immune response. Blood separation allows researchers to study **only the target cell population** without any interference from other cell types



Figure-23

## STORAGE

### WHOLE BLOOD

- whole blood can be refrigerated for up to 35 days, depending on the type of anticoagulant used.
- Whole blood is used to treat patients who need all the components of blood, such as those who have sustained significant blood loss due to trauma or surgery.

### PACKED RED CELL

- Packed red blood cells (PRBCs) have a shelf life of 21–49 days depending on the additive solution used and jurisdiction.<sup>1</sup> During in vitro storage, PRBCs accumulate cellular and biochemical changes collectively called the red blood cell (RBC) storage lesion.
- Typically one unit of PRBCs is approximately 350 mL in volume, of which RBC volume is 200 to 250 mL. , WBCs, platelets, and anticoagulants. The most commonly used anticoagulant is CPDA-1 (citrate, phosphate, dextrose, adenine), which allows for 35 days storage at 1° to 6°C. The hematocrit of such units is less than 80% (range 70% to 80%).

### FRESH FROZEN PLASMA

- It can be prepared either from whole blood or plasmapheresis within 8 h of donation, and it must be stored at  $-18^{\circ}\text{C}$  or colder. The shelf life of FFP is 12 months, but it can be extended to 7 years if stored at  $-65^{\circ}\text{C}$  [2]. FFP contains all stable and labile coagulation factors, such as factor (F) V and FVIII.

## PLATELETS

Platelets (PLTs) are usually stored for up to 5 days prior to transfusion, although in some blood services the storage period is extended to 7 days.



Figure-24

## BLOOD BAG

- ✓ Blood bags are designed for the collection, processing and storage of whole blood and blood components.
- ✓ It acts as a closed system reducing the chances of contamination.

There are 4 types of blood bags. They are

### 1) Single Blood bag

- ❖ For whole blood collection
- ❖ The bag contains CPDA solution
- ❖ Available in capacity of 350ml, and 450ml.

### 2) Double Blood bag

- ❖ For whole blood collection
- ❖ Separation of 2 different blood components (red blood cells and plasma) obtained through the process of Centrifugation and extraction

### 3) Triple Blood bag

- ❖ Triple Blood bag with SAGIM, for Whole blood collection.
- ❖ separation 3 different blood components. (Red blood cells, plasma, platelets)
- ❖ The primary bag contains CPD and One Sterile bag contains SAGM. (Saline Adenine Glucose Mannitol).

### 4) Quadruple Blood bag

- ❖ It Comes with SAGM for Whole blood Collection.

- ❖ Separation for 3 different blood Components (red blood cells, plasma and platelets) though the buffy coat method



Figure-25

## DONOR COUNSELLING

### Two types of counseling

1. Pre donation counseling
2. Post donation counseling

#### **Pre donation counseling**

- ❖ Verify the identification of the donor and ensure that you have the correct Medical History Form.
- ❖ Review the donor information part of the Medical History Form with the donor.
- ❖ Assure the donor that his/her information will be kept confidential.
- ❖ Discuss with the donor risk behavior for HIV and other sexually transmitted diseases.
- ❖ Explain to the donor the consequences of not being honest when answering the questions.
- ❖ Encourage feedback and questions from the blood donor.
- ❖ Explain to the donor the quantity of blood to be drawn, tests to be carried out the donated blood and inform him/her that he/she will be notified of the results if any of the results are irregular
- ❖ Inform the donor about the importance of self-exclusion from the donation process if he/she believes his/her blood is not suitable for transfusion.
- ❖ Discourage blood donation in order to obtain tests results.
- ❖ Review the questionnaire with the donor and ask further questions where the responses are not clear.

#### **Post donation counseling**

- ❖ In the case of a blood donor who has a confirmed TTI reactive result:

- ❖ Inform the donor of the result simply and clearly.
- ❖ Give the donor time to consider the information.
- ❖ Ensure that the donor understands the result.
- ❖ Allow the donor to ask questions.
- ❖ Help the donor cope with emotions arising from the test result
- ❖ Discuss any immediate concerns and assist the donor to suggest a person among their close family and friends who may be available and acceptable to offer immediate support.
- ❖ Describe follow-up services that are available in the health facility and in the community, with special attention to available services for treatment, care and support
- ❖ Provide information on other relevant preventive health measures, such as healthy lifestyles and good nutrition.

## SCREENING

Pre-donation screening of blood donors for Transfusion Transmissible Infections (TTI) is the practice by which a prospective donor is tested for the presence of one or more of the TTI agents by a single rapid or quick method, and donation is deferred if the test is reactive for any of the TTI markers.

## HUMAN IMMUNO DEFICIENCY VIRUS

HIV is a fourth-generation solid phase Enzyme Linked Immunosorbent Assay (ELISA) which employs Agglutinating sera for p24 antigen and highly purified recombinant antigens representing envelope glycoprotein gp 41 of HIV 1 and envelope glycoprotein gp 36 of HIV 2. The use of HIV antigens to sandwich specific antibodies enables detection of both IgG and IgM antibodies. Detection of p24 antigen also reduces window period Present evidence indicates that acquired immunodeficiency syndrome is caused by HIV-1 and HIV-2.

## PROCEDURE

- ✓ Add 50 µl of activated antibody reagent in each well
- ✓ Add 100 ready-to-use substrate & incubate for 30 minutes at RT (22-28 C) µl
- ✓ Add 100 diluted conjugate in each well & incubate for 30 minutes at 37 C µl
- ✓ Add 100 µl control or specimen in the wells
- ✓ Apply plate sealer & incubate for 60 minutes at 37 C Wash six times.
- ✓ Blot dry Wash six times. Blot dry

- ✓ Add 100 µl of stop solution to stop reaction
- ✓ Read results @ 450 nm (ref 600-700nm) within 30 minutes

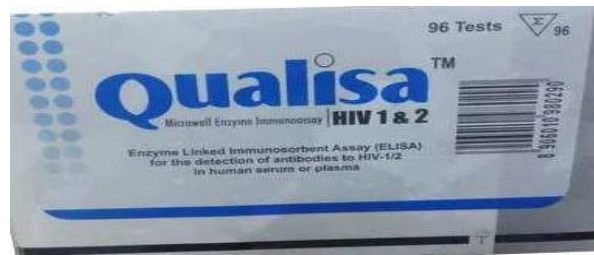


Figure- 28

## HEPATITIS C VIRUS (HCV)

Hepatitis C Virus (HCV) is now known to be the major cause of parenterally transmitted non-A, non-B hepatitis. Until the virus was characterized, diagnosis was made by exclusion of all other known causes of hepatitis. Antibody to HCV is found in over 80% of patients with well documented non-A, non-B hepatitis. The worldwide prevalence of HCV is 0.2 to 2% in blood donors and up to 80% in intravenous-drug users. HCV is an Enzyme Linked Immunosorbent Assay (ELISA) that employs highly purified recombinant antigens representing most conserved antigenic segments of Core, NS3, NS4 and NS5 antigens from multiple genotypes.

### PROCEDURE

- ❖ Add 10 ul of control or specimen in the wells
- ❖ Add 200 ul sample diluent in separate wells
- ❖ Gently shake the plate to mix thoroughly Apply plate sealer & incubate for 30mins @22-28 C
- ❖ Wash six times. Blot dry
- ❖ Add 100 ul diluted conjugate in each well & incubate for 30 minutes @ 22-28 C
- ❖ Wash six times. Blot dry
- ❖ Add 100 ul ready-to-use substrate & incubate in dark for 30 minutes @ 22-28 C
- ❖ Add 100 ul of stop solution to stop reaction
- ❖ Read results @ 450nm (ref:600-700nm) within 30 mins



Figure-27

## HEPATITIS B VIRUS (HBV),

The hepatitis B virus surface antigen (HBsAg) has been shown to be an early marker of infection by the hepatitis B virus (HBV) and therefore, qualitative HBsAg assays like radioimmune assays (RIAs) and enzyme immunoassays (EIAs) have been used as screening tools for several decades.

### Procedure

1. Remove the desired number of strips and secure them in the microwell frame.
2. Reseal un-used strips.
3. Add specimens according to the designation on the ELISA Working Sheet
4. Add 50  $\mu$ L of the HRP- HBsAb conjugates to each well, except the blank well.
5. Gently rock the wells for twenty second, then cover the wells.
6. Incubate the wells at 37°C for to 60 minutes.
7. Carefully remove the incubation mixture by emptying the solution into a waste container. Fill each well with diluted wash buffer and shake gently for 20-30 second.
8. Discard the wash solution completely and tapping the plate on absorbent paper. Repeat above procedure 4 more times.
9. Add 50  $\mu$ L (or 1 drop) of TMB substrate A and 50  $\mu$ L (or 1 drop) of TMB substrate B into each well including the blank well.
10. Incubate at 37°C in dark for 15 minutes.
11. Stop the reaction by adding 50  $\mu$ L (1 drop) of stop buffer to each well. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
12. Set the microplate reader wavelength at 450 nm and measure the absorbance (OD) of each well against the blank well within 15 minutes after adding Stop Solution.



Figure-28

## THE VENEREAL DISEASE RESEARCH LABORATORY TEST(VDRL)

The Venereal Disease Research Laboratory (VDRL) tests are slide micro flocculation test that detect antibodies produced against antigens released by damaged host cells in patients suffering from syphilis.

### PROCEDURE

- Patients' serum is inactivated by heating at 56°C for 30 minutes in a water bath to remove non-specific inhibitors (such as complement
- VDRL antigen suspension (colloidal suspension of tissue cardio lipid or chemically synthesized cardiolipin), controls and samples are brought to room temperature.
- One drop (50 µl) of the test specimen, positive and negative controls is pipetted onto separate reaction circles of the disposable slide.
- A drop of diluted antigen suspension is added to the measured volume of specimen, positive and negative controls.
- Using a mixing stick the test specimen and the VDRL reagent is mixed such that it thoroughly spreads uniformly over the entire reaction circle.
- The slide gently rotated and continuously either manually or on a mechanical rotor at 180 rpm.
- Flocculation is checked microscopically using 10X objective and eye piece at about 8 minutes.



Figure-29

## MALARIA

Five species of Plasmodium parasite are responsible for malaria infection in humans viz P. falciparum, P. vivax, P. ovale, P. malaria and P. knowlesi. This test detects the presence of malaria genus specific pLDH released by parasitized blood cells. Since pLDH is produced by viable TM parasites, the assay can also be used to monitor success of anti-malarial therapy.

## PROCEDURE

- ✓ Add 25 µl control/whole blood specimen in separate wells.
- ✓ Add 100 µl of antibody reagent diluted with sample diluent in each well.
- ✓ Gently shake the plate to mix contents.
- ✓ Apply plate sealer & incubate for 30 minutes at 37°C
- ✓ Wash six times with soak time of 30 sec for each wash. Blot dry
- ✓ Add 100 µl of diluted conjugate in each well and incubate for 30 minutes at 22-28°C
- ✓ Wash six times with soak time of 30 sec for each wash. Blot dry
- ✓ Add 100 µl of ready-to-use substrate. Incubate in dark for 30 minutes at 22-28°C.
- ✓ Add 100 µl of stop solution to stop the reaction in each wells
- ✓ Read the results at 450 nm (ref: 600 – 700 nm) within 30 minutes



Figure-30

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## CHENGALPATTU MEDICAL COLLEGE -INTERNSHIP CERTIFICATES



**CHENGALPATTU MEDICAL COLLEGE, CHENGALPATTU - 603001**  
(AFFILIATED TO THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY,  
CHENNAI-600032.)




**GOVT. CHENGALPATTU MEDICAL COLLEGE, CHENGALPATTU 603001**  
**TAMILNADU**  
**CERTIFICATE OF OBSERVERSHIP**

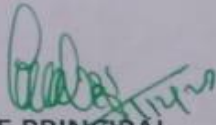
This is to certify that the following students studying in Sathyabama Institute of Science and Technology, Chennai has undergone observership for a period of One Month From 01.11.2023 & 30.11.2023 in the Department of Microbiology & Histopathology at Chengalpattu Medical College, Chengalpattu-603001.

| Sl.No. | Name of the Student | Department                          | Period of posting              |
|--------|---------------------|-------------------------------------|--------------------------------|
| 1      | Mary G              | Microbiology<br>&<br>Histopathology | 01.11.2023<br>to<br>30.11.2023 |
| 2      | Stephen Xavier M    |                                     |                                |
| 3      | Oviya M.S           |                                     |                                |
| 4      | Monika G            |                                     |                                |
| 5      | Sowmiya S           |                                     |                                |
| 6      | Shakeera Banu K     |                                     |                                |
| 7      | Beruthose Banu S    |                                     |                                |
| 8      | Ajith C             |                                     |                                |
| 9      | Santhosh Kumar M.S  |                                     |                                |

They attend the training during the above period has been Good.



Date:21.12.2023  
Place:Chengalpattu.



**VICE PRINCIPAL**  
Chengalpattu Medical College,  
Chengalpattu.

8-12-2023

## BLOOD BANKING- CERTIFICATE

*Sharing Life. Saving Lives..since 1984*



**Lions BloodBank**  
Tollfree HelpLine **1910**

**Madras Egmore  
Lions Blood Bank &  
Research Foundation**

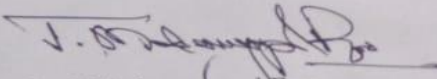
Date: 12<sup>th</sup> Dec 2022

**TO WHOMSOEVER IT MAY CONCERN**

This is to certify that Ms. Mary. G, BSc in MLT, 2nd year of Sathyabama Institute of Science and Technology, Chennai - 119, undergone Internship training in Madras Egmore Lions Blood Bank & Research Foundation, Egmore, Chennai - 08. She has been observed blood bank procedures including Bleeding the Donors, Bloodgrouping, Cross-matching, Component separation, TTI screening and attend Blood donation camp for the period from 21<sup>st</sup> Nov 2022 to 26<sup>th</sup> Nov 2022

During her internship, performance was good and she was able to complete the project on time.

We wish him all the best for her upcoming career.



Ln.J.Madanagopal Rao  
Chairman



This project made possible through the co-operation of Lions Club International Foundation, Clubs in the District 324 - A1, A5 & A6 and well wishers - sponsored by Lions Club of Madras Egmore

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Web : [www.lionsbloodbank.net](http://www.lionsbloodbank.net)  
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Mob : 99419 17505  
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## **CONCLUSION**

My internships at both **CHENGALPATTU MEDICAL COLLEGE – CHENGALPATTU DISTRICT and EGMORE LIONS BLOOD BANK AND RESEARCH FOUNDATION- EGMORE** has been great learning experience. During this internship I have acquired knowledge in these techniques and attained comprehensive knowledge regarding the tests conducted in clinical laboratories. Through this learning process, I have developed a deeper understanding of laboratory procedures and its importance in health care.